Structure of the Capsid of Pf3 Inovirus Determined from 3.1 Å Fibre Diffraction Data

L.C.Welsh, M.F.Symmons, D.A.Marvin and R.N.Perham

Cambridge Centre for Molecular Recognition, Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge, CB2 1GA, U.K.

Many Inovirus (filamentous bacteriophage) virions have been isolated and all share certain structural and biological features. The virions are between 800 and 2000nm in length and about 6nm in diameter, depending on the biological strain. They comprise a semi-flexible helical tube of several thousand copies of a major coat protein subunit surrounding a core of single-stranded circular DNA. The major coat protein subunits of virions of different strains of Inovirus are largely alpha-helical, but have quite different amino-acid sequences. However, the sequences have a similar domain structure, with acidic residues near the N-terminus, a continuous stretch of about 20 apolar residues near the centre of the sequence, and a collection of basic residues near the C-terminus. The N-termini of the major coat protein subunits are on the outside of the capsid and the C-termini are on the inside where they interact with the viral DNA. The virions are held together by hydrophobic interactions between the apolar domains of neighbouring subunits. The virions infect Gram-negative bacteria and are extruded through the bacterial membrane during the subsequent virion assembly process.

Two Inovirus capsid helical symmetry classes have been identified from fibre diffraction patterns: class I includes strain fd; class II includes strain Pf1. Pf1 virions undergo a reversible temperature-induced phase transition at about 10°C between a higher (Pf1H) and a lower (Pf1L) temperature helical symmetry form . Very well-resolved fibre diffraction patterns from Pf1 at temperatures below 10°C enabled many Bessel-function terms that overlap in the higher temperature form diffraction pattern to be separated. Accurate intensity measurements for native and heavy-atom derivatives therefore resulted in a diffraction data set as extensive as a single crystal diffraction data set at the same resolution (4.0Å in R and 2.5Å in Z). These data enabled precise atomic models to be built for the Pf1 major coat protein [1]. Models of class I virions have been built from these that are consistent with the much more limited fibre diffraction data available from class I virions [2].

Pf3 Inovirus has a particularly intriguing structural feature: the Pf3 major coat protein has two basic residues near the C-terminus, like Pf1, but has more than twice as much DNA per unit length. Knowledge of the virion structure is essential for an understanding of the assembly process, the infection process, and DNA/protein interactions within the virion. Similarities and differences between strains help to define the essential aspects of these processes. Therefore, we recorded high resolution X-ray fibre diffraction data from Pf3 and used these data to determine the structure and interactions of the major coat protein subunit within the virion.

X-ray diffraction data recorded from magnetically aligned fibres of virions of the Pf3 strain of Inovirus show that the Pf3 capsid has the same, class II, helical symmetry as that of the higher temperature form of the Pf1 virion. A quantitative comparison of the integrated layer-line intensities extracted from Pf3 (Figure 1) and Pf1 diffraction patterns, using CCP13 software, gives a correlation coefficient of 0.89, showing that the structures of the Pf3 and Pf1 capsids are very similar. However, Pf3 virions do not undergo a temperature-induced helical symmetry transition of the kind observed for Pf1 virions [3].

We have determined the structure of the Pf3 major coat protein within the capsid by a form of molecular replacement from a model of the major coat protein of the higher temperature form of Pf1. A 5Fo-4Fc Fourier synthesis electron density map of the Pf3 capsid was calculated from the Pf3 fibre diffraction amplitude data by separating overlapping Bessel function terms and taking phases from an all alpha-helix model of the Pf1 major coat protein, stripped of side-chains atoms. A set of initial atomic models of the Pf3 major coat protein covering a discrete range of azimuthal major coat protein subunit orientations within the capsid was built, consistent with both the observed fibre diffraction data and the initial Pf3 major coat protein electron density map.

We were able to discriminate between some of the models of the Pf3 capsid on the basis of the sequence

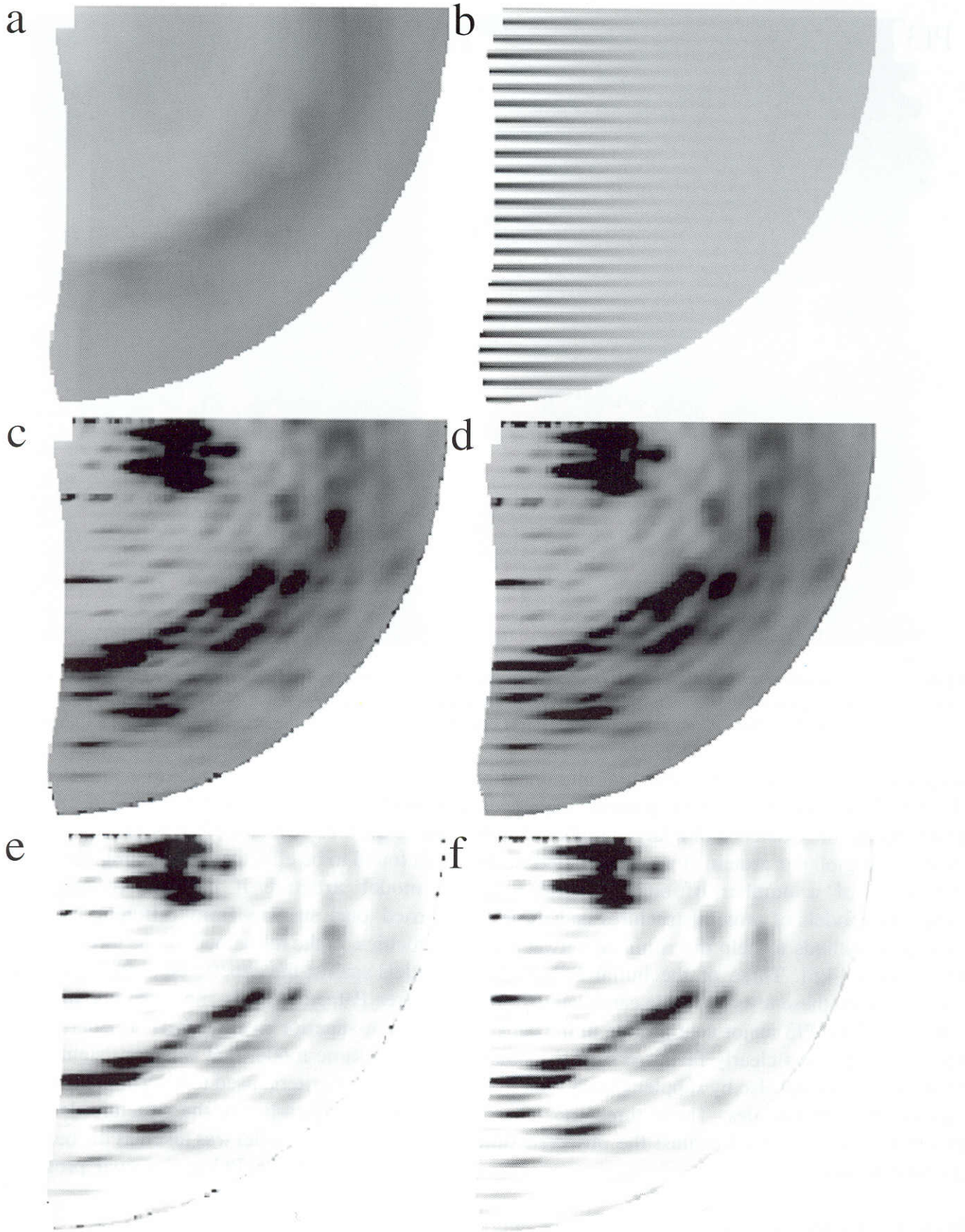

Figure 1: Stages in the processing of the Pf3 fibre diffraction pattern using CCP13 software. The diffraction pattern was recorded on a MAR image plate on station 7.2 of the SRS at CLRC Daresbury. The outer radius of is at 0.32Å^{-1}. The c repeat is 76.2Å. (a) The background scattering calculated from the experimental Pf3 diffraction pattern using the roving window method in the LSQINT program. (b) An image of the layer-line profiles [8] generated by LSQINT to fit the layer-line intensity distributions, before being fitted to the observed diffraction pattern. The disorientation is 2.0° and the coherence length is 200Å. (c) The observed diffraction pattern mapped into reciprocal space and quadrant averaged using the FTOREC program. (d) A simulated diffraction pattern using the integrated layer-line intensity distributions generated by LSQINT from the observed diffraction pattern in (c) and the background scattering shown in (a). (e) The observed diffraction pattern of (c) with the calculated background scattering of (a) subtracted. (f) The simulated diffraction pattern of (d) without the calculated background scattering. The R-factor calculated between the image and that shown in (e) is 0.21.

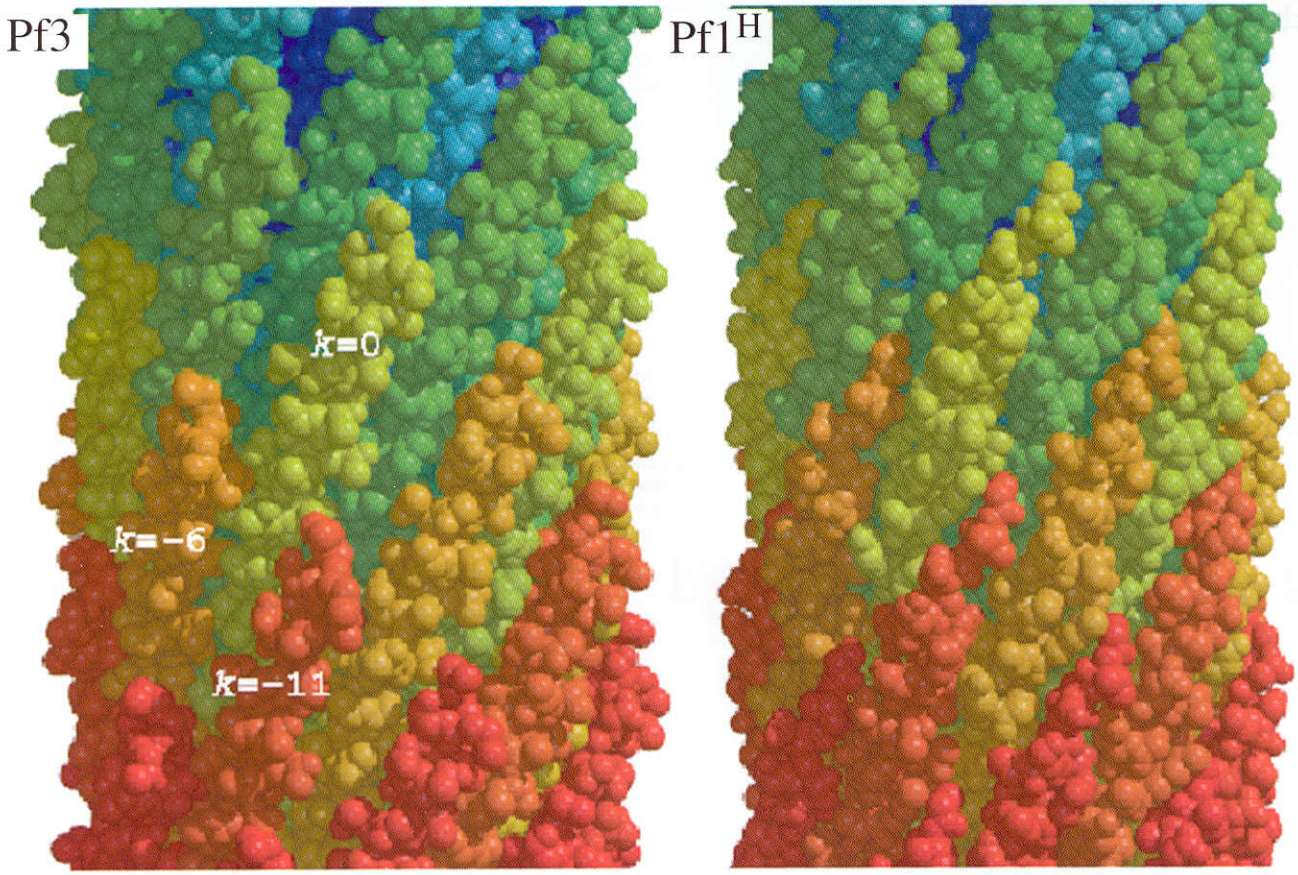

Figure 2: Space-filling representation of a 100Å long section of the final model of the Pf3 capsid and the current model of the higher temperature form of the Pf1 capsid. Side-view of the capsids, virion axis vertical, N-termini of the major coat protein subunits towards the top. The subunit indices of the nearest neighbours of a subunit arbitrarily indexed as 0 are shown.

homology resulting from the structural alignment of the Pf3 and Pf1 major coat protein sequences. Furthermore, the existence of a large number of well determined globular protein structures has led to the derivation of a number of empirical rules for validating models of protein structures and we used two such methods to help discriminate between the Pf3 models [4,5]. Three of the initial Pf3 models emerged from these tests as viable candidates for the structure of the Pf3 major coat protein in the capsid, but one model was clearly favoured over the others by these criteria and also by supplementary data from spectroscopic techniques. These three most likely Pf3 models were refined against the observed fibre diffraction data.

Both Cartesian co-ordinate and torsion angle coordinate simulated annealing refinement protocols were tested by simulation for model refinement against continuous transform fibre diffraction data of the kind obtained from Pf3 virions. Refinement using torsion angle dynamics reduces the number of degrees of freedom of the models [6], but was found to have a lower radius of convergence for Pf3 models refined against fibre diffraction data; models were therefore refined using Cartesian coordinate simulated annealing. In addition, model refinement against continuous transform fibre diffraction data using the free R-factor [7] was tested by simulation and found to be effective in detecting global errors in a model structure if multiple refinements were carried out, starting from different initial atomic velocities. In the refinement of the three potential Pf3 models against the experimental Pf3 diffraction data the free R-factor clearly indicated one of the models as being superior to the others. Encouragingly, this was the same model that was consistently favoured by the other criteria used to discriminate between the initial models earlier in the structure determination procedure. This model was taken as the best model of the structure of the Pf3 major coat protein in the capsid.

The model of the Pf3 capsid thus determined shows that the overall structure of the capsid is very similar to that of Pf1 (Figure 2). Both capsids possess similar networks of non-polar inter-subunit van der Waals contacts between side-chains of residues in the central regions of the major coat proteins. However, there are significant differences in the structures of the N and C-termini of the proteins (Figures 2 and 3). In particular the N-terminus of the Pf3 major coat

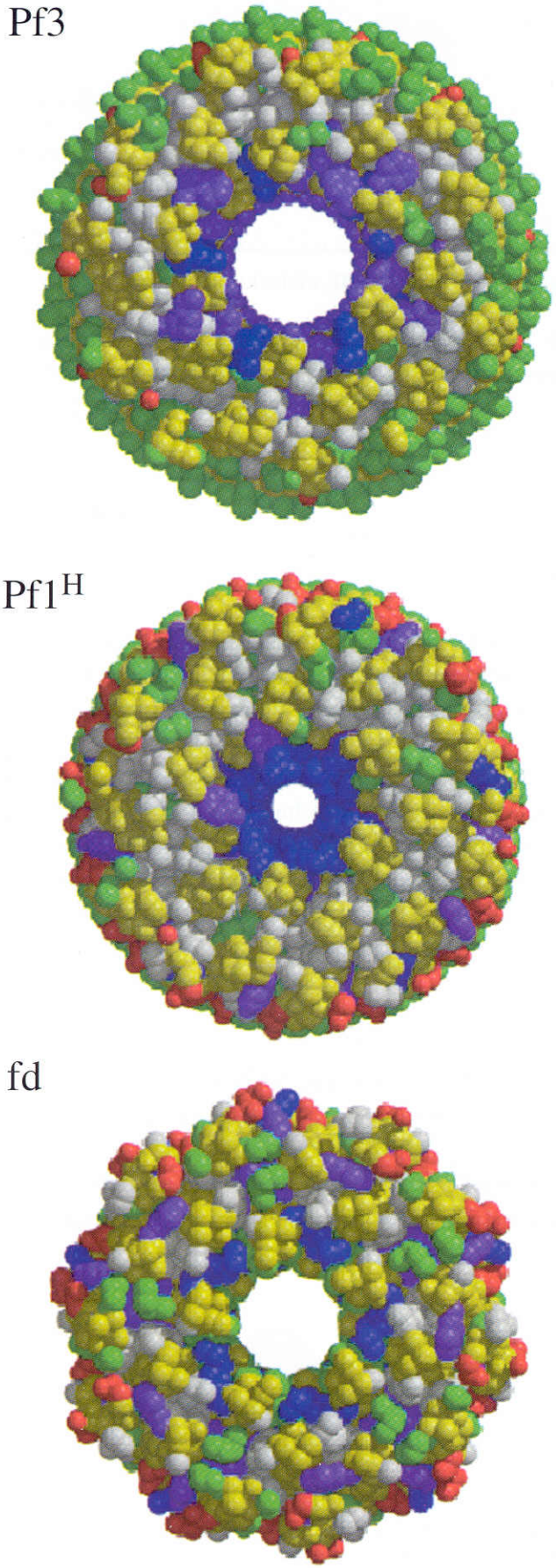

Figure 3: Space-filling representations of models of Inovirus capsids. View down the long axis of the capsids showing the central core occupied by the single-stranded circular DNA (for which there are currently no reliable atomic models). Backbone atoms are coloured yellow, acidic side-chains are coloured red, polar side-chains are coloured green, apolar side-chains are coloured white, aromatic side-chains are coloured purple, and basic side-chains are coloured blue. Models of the Pf3, Pf1H and the fd capsid. The average diameter of the Pf3 core is 25Å, that of fd is 22Å and both have about two and half times as much DNA per unit length as Pf1 which has an average diameter of about 10Å. The maximum radius of the Pf3 models is 33Å and that of the Pf1H models is 30Å, consistent with differences in the equatorial diffraction data from these strains. The maximum radius of the fd model is 31Å.

protein is in an alpha-helical conformation whereas the first five N-terminal residues of Pf1 are not [1]. The nature of the C-terminal region of the inner core of the model of the Pf3 capsid shows that there must be significant DNA/protein interactions in this virion involving both charged side-chains and aromatic side-chains, whereas the DNA/protein interactions in the models of the Pf1 and fd virions predominantly involve charged side-chains (Figure 3). This difference in DNA/protein interactions may result in differences in the low-frequency dynamics of the virions and thereby be responsible for the absence of a temperature transition in the Pf3 virion.

Acknowledgements

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